# ELEVATED CYCLIC GMP CONCENTRATIONS DURING ESTROGEN INDUCED DIFFERENTIATION OF THE CHICK OVIDUCT

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SUMMARY

The levels of cGMP and cAMP in the chick oviduct were evaluated during estrogen-induced differentiation and growth at two different stages of development. In the differentiating oviduct cGMP levels were found to change markedly. Tissue concentrations were elevated 35-fold by 2 hr after primary stimulation and about 3-fold at later time intervals. In contrast, constant levels of cGMP were observed during growth of the differentiated oviduct after secondary stimulation. cAMP concentrations were not or very little changed during differentiation and secondary stimulation of the oviduct. Our data suggest that elevated cGMP levels represent an active signal for the differentiative response of the chick oviduct to estrogens.

Considerable evidence has been obtained for involvement of cAMP[1] (and to a lesser extend of cGMP) in the response of various cellular functions to stimulation by proteohormones. However, there are only a few reports on cyclic nucleotide concentrations and metabolism as a result of steroid hormone administration (1). The chick oviduct represents a useful model for studying steroid hormone induced organ differentiation and cell proliferation in vivo. About 6 months after hatching, rising estrogen levels in the chick elicit oviduct differentiation. In immature animals, differentiation can be induced precociously by DES or several other estrogens (2,3). The cyto-

[1] Abbreviations: cAMP, cyclic 3':5' adenosine monophosphate; cGMP, cyclic 3':5' guanosine monophosphate; DES, diethylstilbestrol.

logical and biochemical events during oviduct differentiation have been studied extensively (4). In particular, it has been shown that the induction of egg-white proteins such as ovalbumin and lysozyme requires the interaction of the steroid hormone-receptor-complex with nuclear constituents and functions. In order to study the response leading to the induction of egg-white proteins without interference of the differentiative response, estrogens have been administered to chicks withdrawn for two weeks from an earlier DES treatment. A secondary stimulation of the differentiated oviduct in these animals results in the rapid synthesis of mRNA sequences specifying egg-white proteins (5,6) and within 24 hr in an increase in cell division (7). In the present study we report on the concentrations of cyclic nucleotides during the differentiative response of the chick oviduct to estrogens as well as during secondary stimulation of the differentiated tissue.

## MATERIALS AND METHODS

### Isotopes and chemicals

$^{3}$H-Labeled cAMP (27.5 Ci/m mol) and $^{3}$H-labeled cGMP (15.0 Ci/m mol) were purchased from Amersham Buchler, Braunschweig. cAMP and cGMP were obtained from Boehringer, Mannheim, and DES from Sigma Chemical Co.

### Treatment of animals

4-Day and 30-day old female HNI-chicks were obtained from Schmidt-Ankum Farms, Ankum, and maintained in electrically heated brooders on a standard chow (Club-Kraftwerke, Hamburg) and tap water. 30-Day old chicks received subcutaneous injections of 2.5 mg DES dissolved in 0.2 ml of sesame oil and were sacrificed after specified time intervals by cervical dislocation. Control animals received sesame oil only. The oviduct was quickly removed and immediately frozen in liquid nitrogen. Removal and freezing of the oviduct were accomplished within 20 seconds. Tissue was stored in liquid nitrogen for up to 4 weeks prior to homogenisation. No change in cAMP and cGMP levels was obtained when tissues were homogenized immediately or after storage up to 4 months.

Daily subcutaneous injections of 2.5 mg DES were administered to 4-day old chicks over a period of 14 days, after which they were withdrawn from hormone treatment for two weeks. Then, they received a single subcutaneous injection of 2.5 mg DES and were sacrificed after indicated time intervals.

Assay for cyclic nucleotides

Cyclic nucleotides were extracted by homogenizing frozen oviduct tissues in glass-glass homogenizers with ice-cold 5% trichloroacetic acid. Because of the small size of the undifferentiated oviduct (about 40 mg), tissues from ten 30-day old chicks, which received exactly the same treatment, were pooled and extracted with 4 ml trichloroacetic acid. Differentiated oviduct tissues from withdrawn chicks, weighing about 0.2 g, were homogenized each in 3.5 ml trichloroacetic acid. The homogenates were centrifuged at 15.000 x g for 15 min at 4°C. The supernatant was decanted and extracted five times with five volumes of water saturated ether. To remove residual ether the aqueous phase was heated 4 min at 95°C. Standards with known concentrations of cyclic nucleotides were processed identically. Aliquots of 100-200 µl of different dilutions were assayed for cAMP and cGMP content by the competition radioimmunoassay following the method of Steiner et al. (8,9) (antisera prepared by M.S.). Each sample was run in triplicate. Variations of cAMP and cGMP determinations were 0-5% for intra-assay measurements. The inter-assay variations varied between 2 and 9%. The relative cross-reactivity of cGMP was 0.003% in the cAMP-assay and of cAMP 0.0005% in the cGMP-assay. The lowest detectable dose of both nucleotides was below 50 femto moles.

## RESULTS

The levels of cAMP and cGMP were evaluated during estrogen-induced differentiation of the oviduct in immature chicks (primary stimulation) and during a secondary stimulation of the differentiated organ in withdrawn chicks. In control immature chicks, oviduct tissue levels for cAMP and cGMP were 789 ± 37 and 18.9 ± 4.6 pmol/g wet weight, respectively. The changes in cyclic nucleotide concentrations of the differentiating oviduct at time intervals after DES administration to immature chicks are shown in Fig. 1. Only a moderate decline of cAMP levels was observed at 1.5, 2, and 4 hr after DES injection. Sham-injected chicks showed no change of oviduct cAMP content at 0.5, 2, and 4 hr. In contrast, the cGMP level in the undifferentiated ovi-

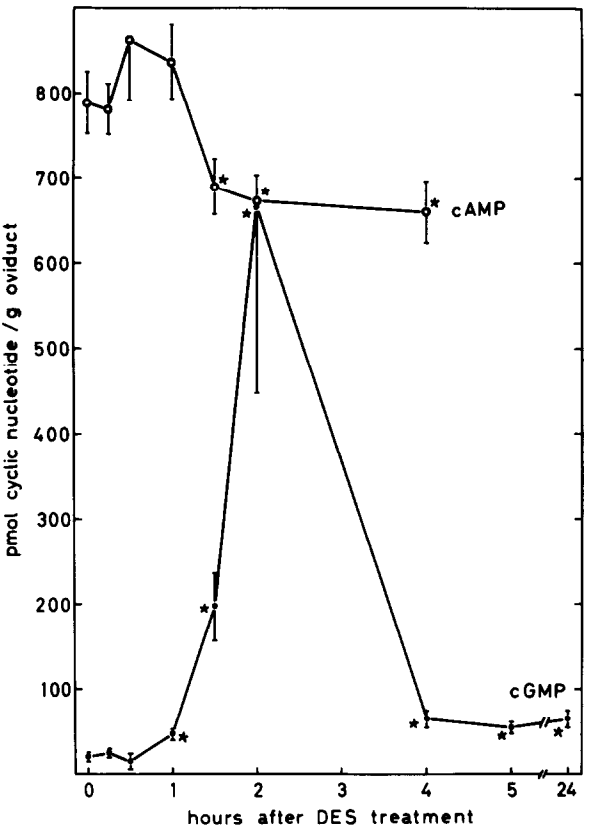


Figure 1. Changes in cyclic nucleotide levels during primary stimulation of the undifferentiated oviduct. cAMP (o—o) and cGMP (•—•) concentrations were measured at various time intervals after a single administration of DES to 30-day old immature chicks. Each point represents the mean ± SEM of two to three groups consisting each of 10 chicks. Astericks denote values that are significantly different from their respective controls ($P<0.05$).

duct had increased to 253% of control within 1 hr after DES injection and then it further rose sharply to a 35-fold concentration at 2 hr. In some experiments, the cGMP level exceeded the cAMP level transiently during differentiation. At 4 hr after hormone treatment, the cGMP level had returned to about 300% of control, where it remained at 5 and 24 hr. Sham-injected animals showed no change of oviduct cGMP content at 2 and 8 hr.

The effects of a single DES injection on cyclic nucleotide

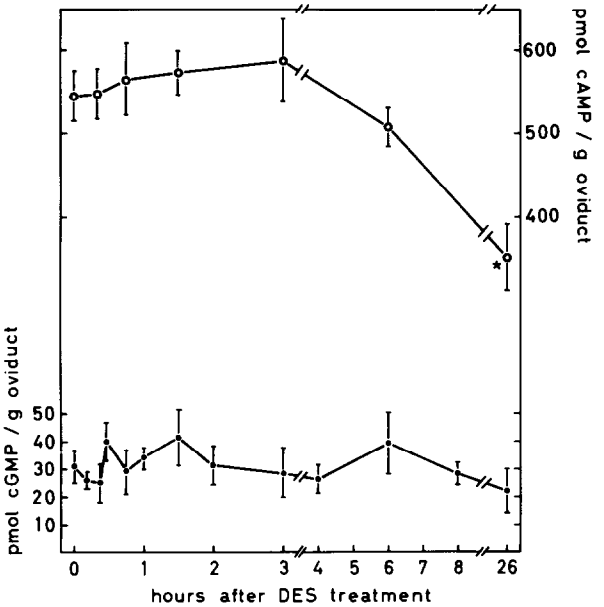


Figure 2. Changes in cyclic nucleotide levels during secondary stimulation of the differentiated oviduct. Withdrawn chicks were restimulated by a single injection of DES after which cAMP (o—o) and cGMP (●—●) levels were measured at various time intervals. Each point is the mean ± SEM of two to three chicks. Astericks denote values that are significantly different from their respective controls ($P < 0.05$).

levels in the differentiated oviduct of withdrawn chicks (secondary stimulation) are shown in Fig. 2. In control chicks, oviduct tissue levels for cAMP and cGMP were 544 ± 30 and 31.2 ± 6.1 pmol/g wet weight, respectively. cAMP concentrations were not significantly different up to 6 hr after DES treatment and then decreased to 65% of control at 26 hr. Sham-injected chicks showed no change in oviduct cAMP concentration at 26 hr. cGMP levels were not significantly changed at any time interval after restimulation.

## DISCUSSION

Only very small changes in the cAMP concentration were observed after primary and secondary stimulation of the chick oviduct. These results are consistent with previously published

reports (10,11). The cAMP level in the undifferentiated oviduct was somewhat higher than in the differentiated tissue from withdrawn chicks. This probably reflects that undifferentiated cells had differentiated into three distinct cell types (tubular gland cells, goblet cells, and ciliated cells) (3). The decrease of cAMP concentration at 26 hr after DES treatment during secondary stimulation is likely to result from an increase in the water content of the tissue giving to a higher wet weight.

In contrast to the moderate decrease of cAMP, cGMP levels underwent marked changes during the early period of differentiation. Within 2 hr after primary stimulation, the cGMP level rose about 35-fold. At later time intervals after DES administration, cGMP concentration declined and remained at a level about three times higher than in the undifferentiated tissue. During secondary stimulation of the differentiated oviduct in withdrawn animals, however, the cGMP level remained constant with respect to wet weight. It is known that oviduct growth resumes rapidly after DES administration to withdrawn chicks (2,7) and an increase in cell division occurs within 24 hr (7). Therefore, the contrast between the drastic changes in cGMP during primary stimulation and the constant level during secondary stimulation suggests to us that elevated cGMP levels represent an active signal for the onset of differentiation in the chick oviduct. As the cAMP level was constant or slightly decreased during the early period of differentiation, the cAMP concentration may play a passive role in the differentiative process. In contrast to the differentiative response of the immature oviduct, stimulation of growth and secretory functions in the differentiated tissue appears not to involve changes of the cGMP level.

The marked increase in cGMP during estrogen induced differ-

entiation, along with recent progress in the elucidation of steroid hormone action (4-6), recommends the chick oviduct as a useful model for studies of the regulatory role of cGMP. It has been reported that cGMP levels vary markedly during development of the insect Ceratitis capitata (12) and during differentiation of the fungus Blastocladiella emersonii (13). Furthermore, elevated cGMP levels were monitored during erythropoiesis in several systems (14). These studies suggest that changes in cGMP concentration are involved also in other differentiating systems.

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